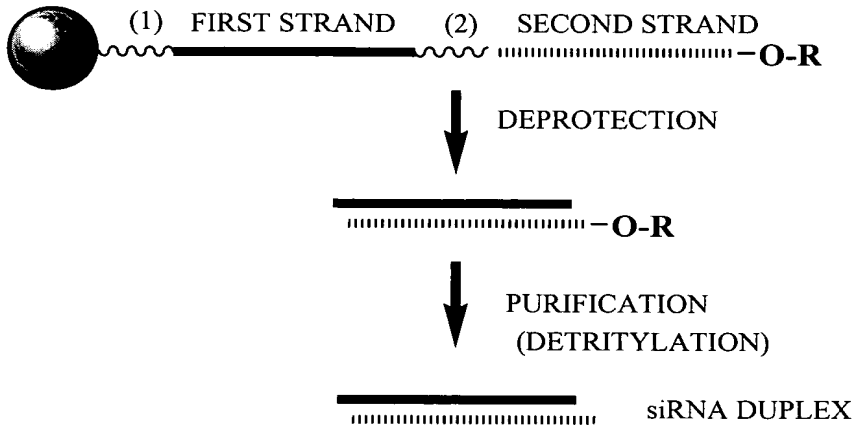
Figure 1

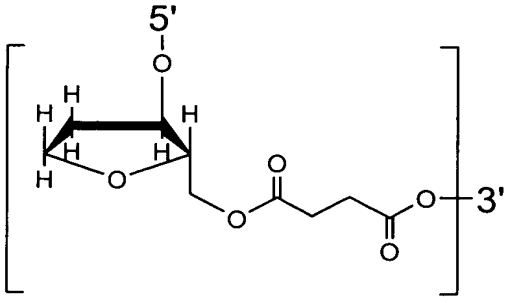


= SOLID SUPPORT

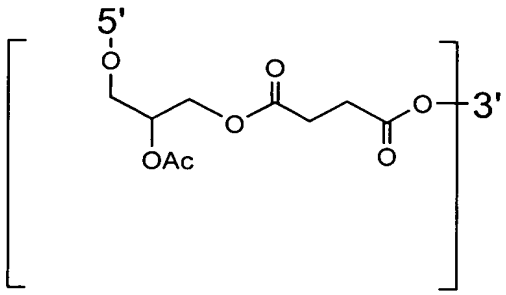
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

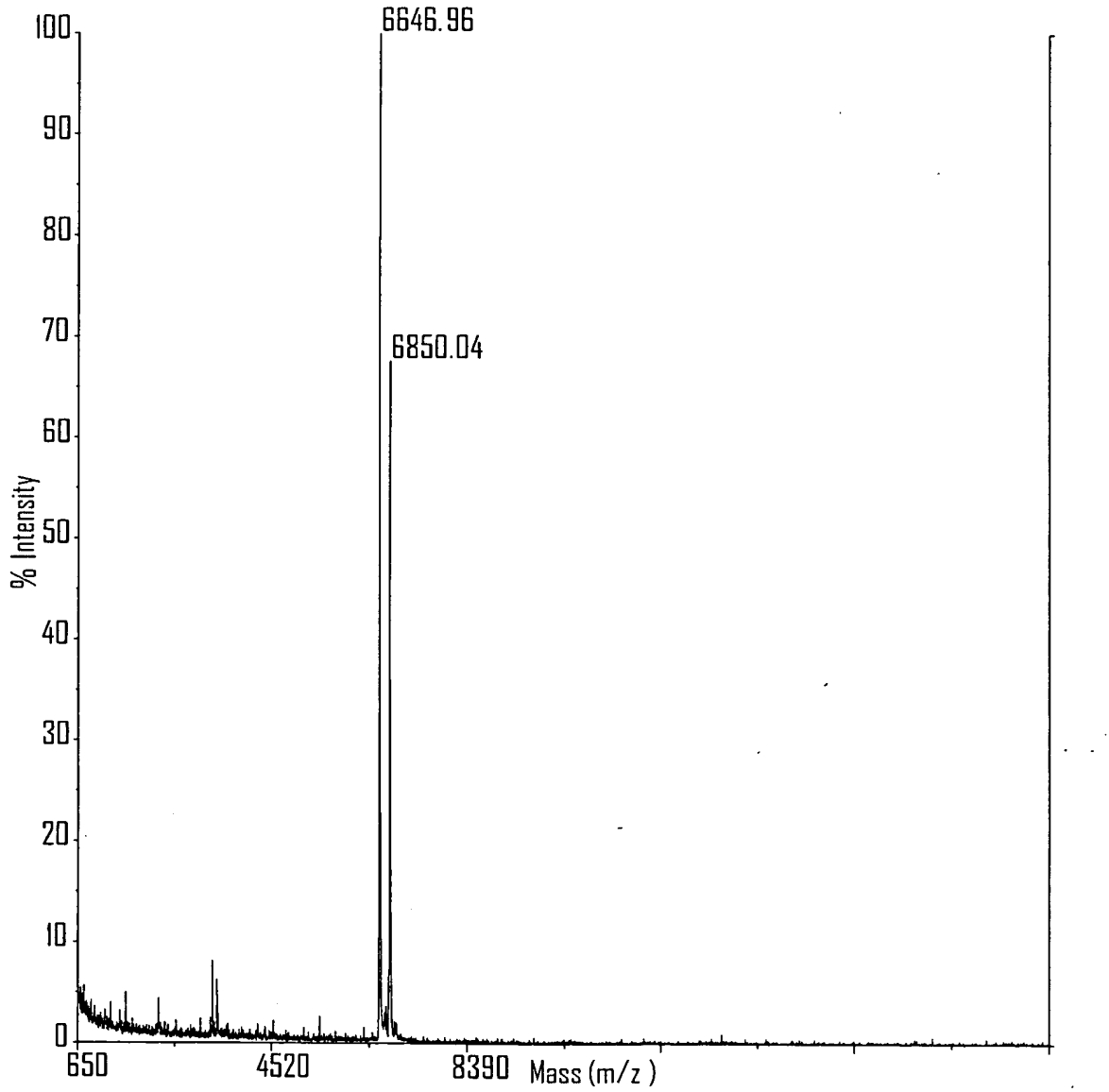

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

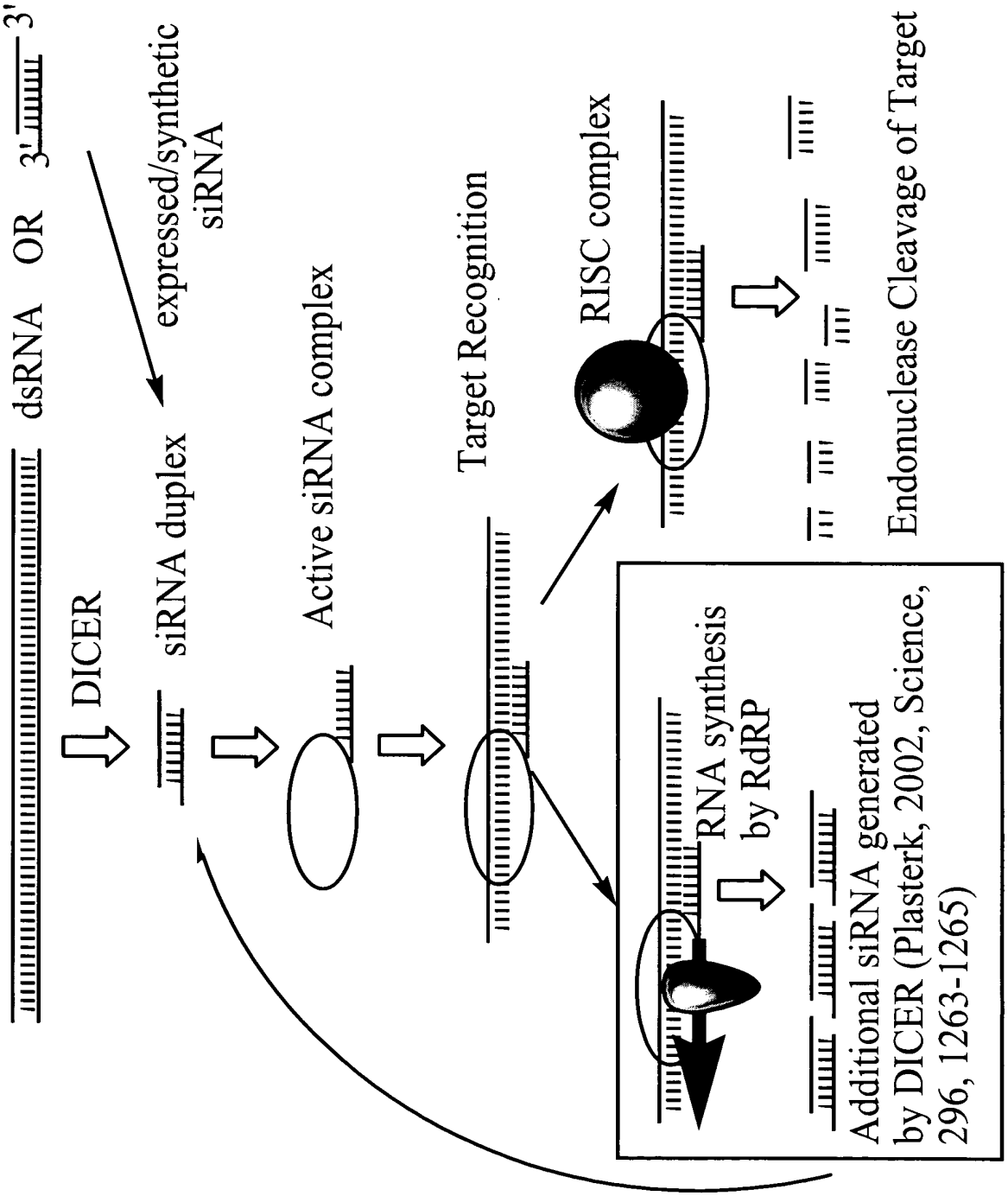

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 4

A

SENSE STRAND (SEQ ID NO 2554)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2555)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 2556)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_SN) -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2557)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 2558)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2559)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 2560)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2557)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 2561)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2557)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 2560)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2562)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5

A

SENSE STRAND (SEQ ID NO 2563)

5'- iB- U C U G A U G A U G U C A G A U A U G *T T*-iB -3'
3'- L-T_S*T* A G A C U A C U A C A G U C U A U A C -5'

ANTISENSE STRAND (SEQ ID NO 2564)

B

SENSE STRAND (SEQ ID NO 2565)

5'- *u c u* g a *u* g a *u g u c* a g a *u* a *u* g T_S*T* -3'
3'- L-T_S*T* a g a *c u* a *c u* a *c* a g *u c u* a *u* a *c* -5'

ANTISENSE STRAND (SEQ ID NO 2566)

C

SENSE STRAND (SEQ ID NO 2567)

5'- iB-u c u G A u G A u G u c A G A u A u G *T T*-iB -3'
3'- L-T_S*T* A G A *c u* A *c u* A *c* A G *u c u* A *u* A *c* -5'

ANTISENSE STRAND (SEQ ID NO 2568)

D

SENSE STRAND (SEQ ID NO 2569)

5'- iB-*u c u G A u G A u G u c A G A u A u G T T*-iB -3'
3'- L-T_S*T* a g a *c u* a *c u* a *c* a g *u c u* a *u* a *c* -5'

ANTISENSE STRAND (SEQ ID NO 2566)

E

SENSE STRAND (SEQ ID NO 2570)

5'- iB-*u c u* G A *u* G A *u* G *u c* A G A *u* A *u* G *T T*-iB -3'
3'- L-T_S*T* a g a *c u* a *c u* a *c* a g *u c u* a *u* a *c* -5'

ANTISENSE STRAND (SEQ ID NO 2566)

F

SENSE STRAND (SEQ ID NO 2569)

5'- iB-*u c u G A u G A u G u c A G A u A u G T T*-iB -3'
3'- L-T_S*T A G A c u A c u A c A G u c u A u A c* -5'

ANTISENSE STRAND (SEQ ID NO 2571)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro
italic lower case = 2'-deoxy-2'-fluoro
underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
iB = INVERTED DEOXYABASIC
L = GLYCERYL MOIETY or iB OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

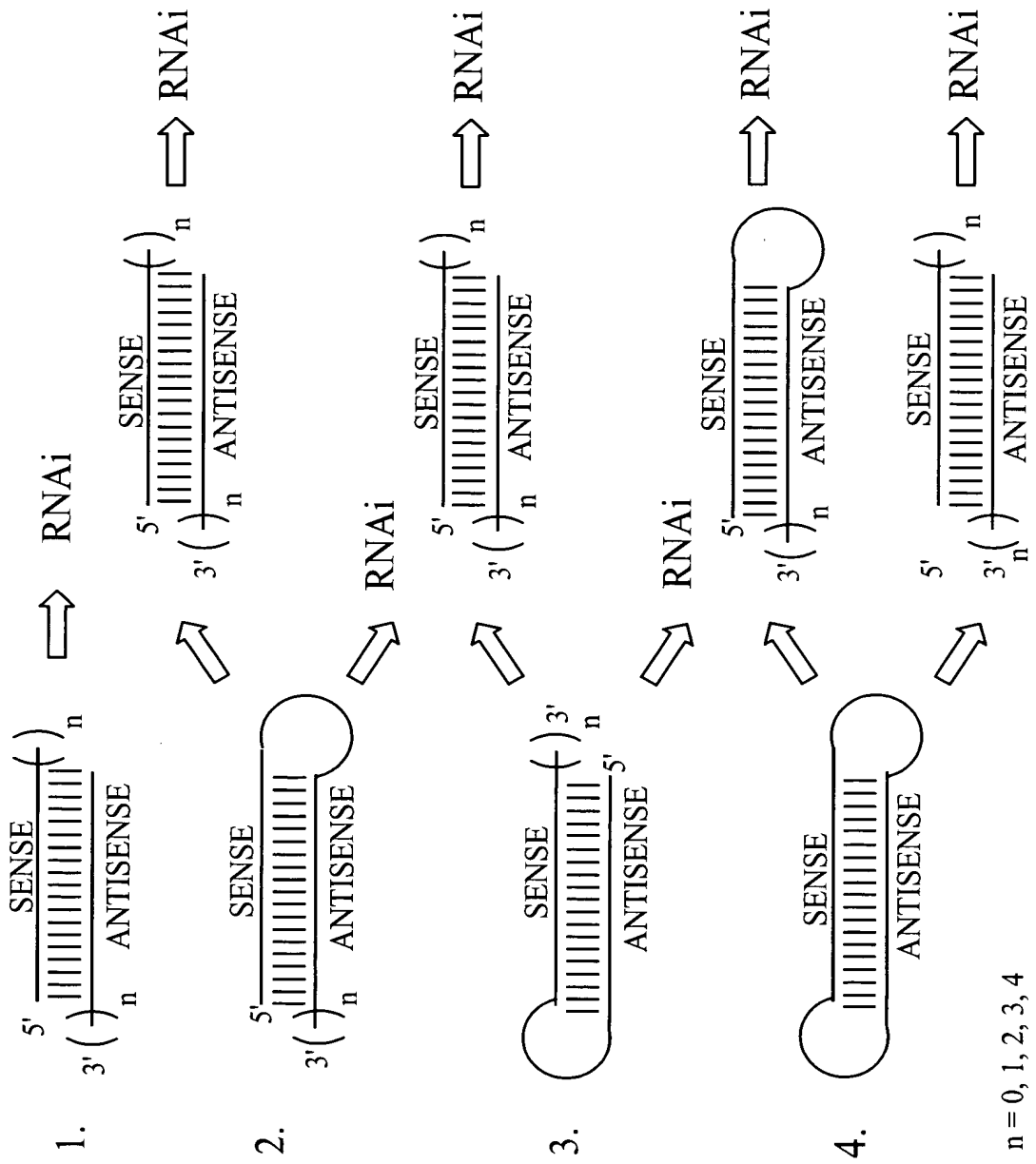


Figure 7

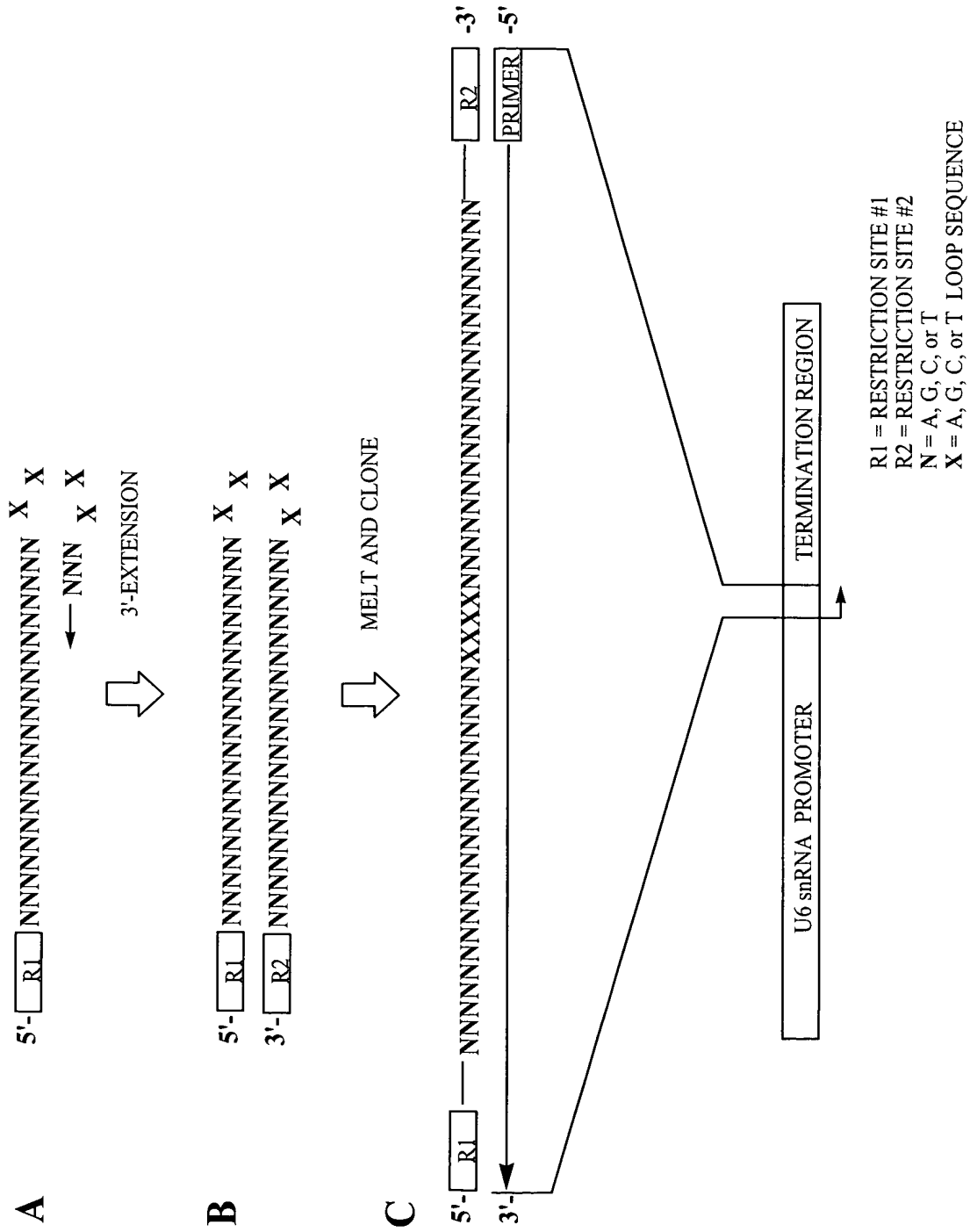

A
5'- R1 NNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 — NNNNNNNNNNNNNNNNNNNXXXNNNNNNNNNNNNNNNNNNN — R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8

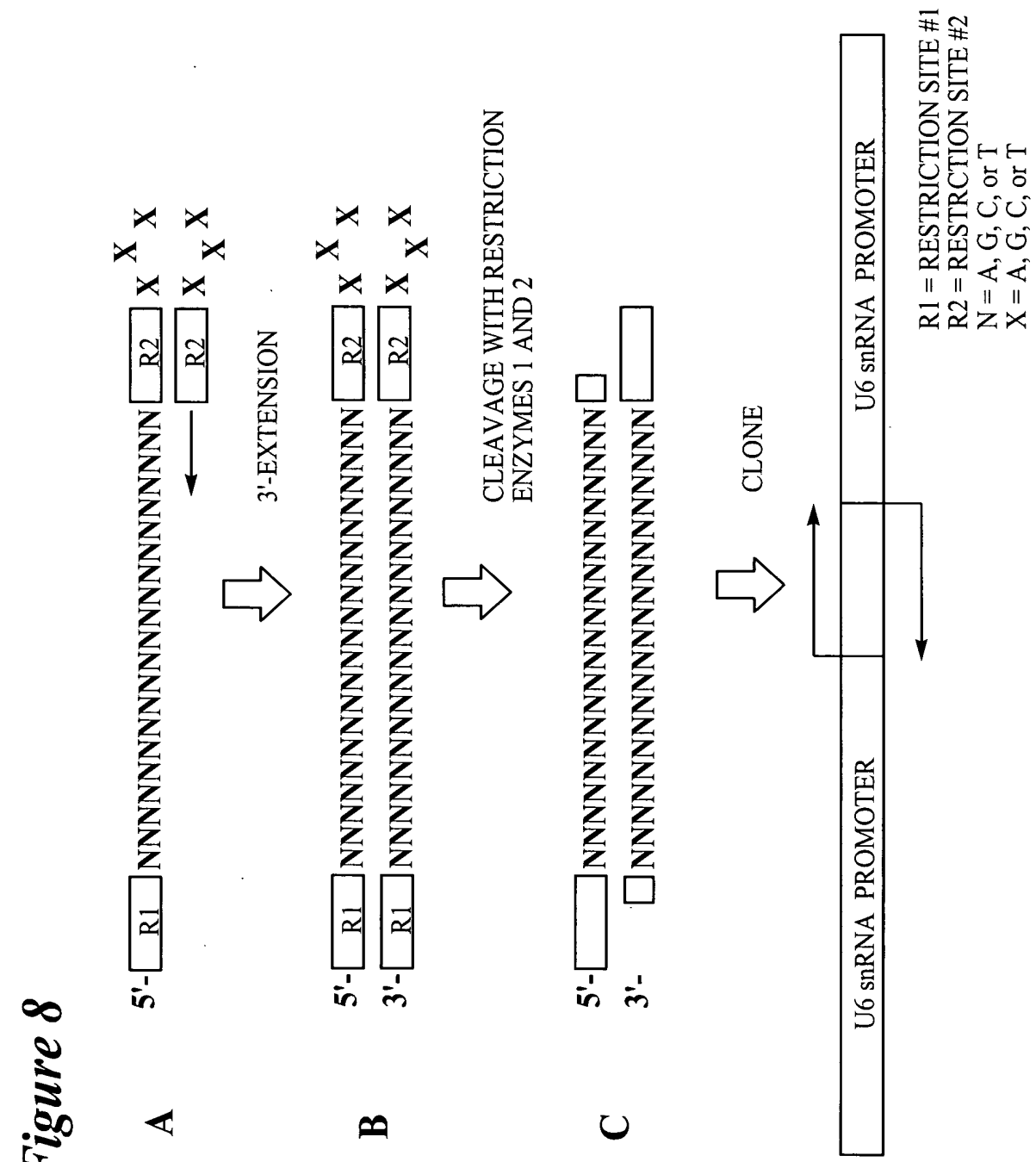

A
5'-
R1
NNNNNNNNNNNNNNNNNNN
R2
X X
X X
X X
3'-EXTENSION
B
5'-
3'-
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

Figure 9: Target site Selection using siRNA

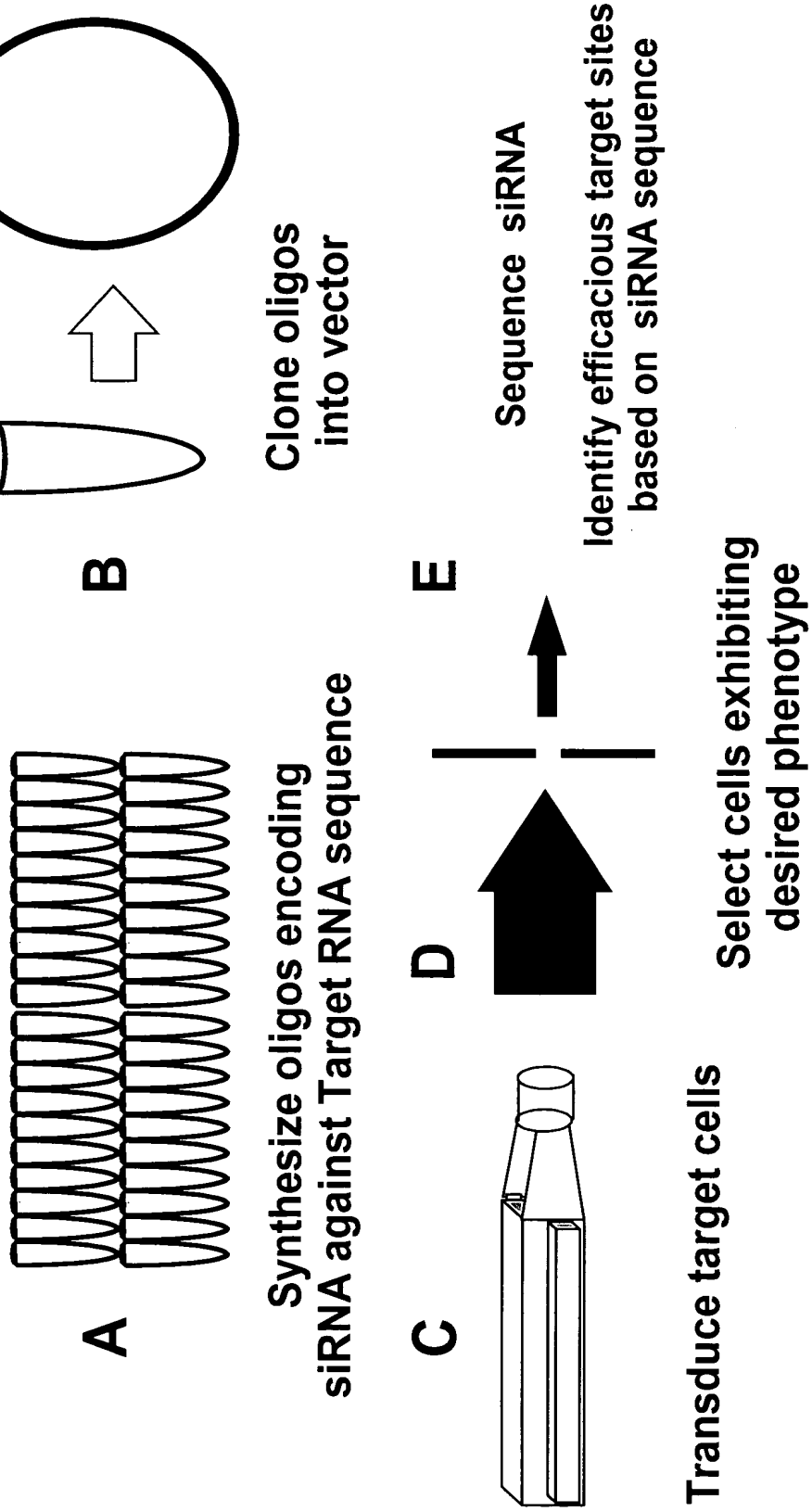
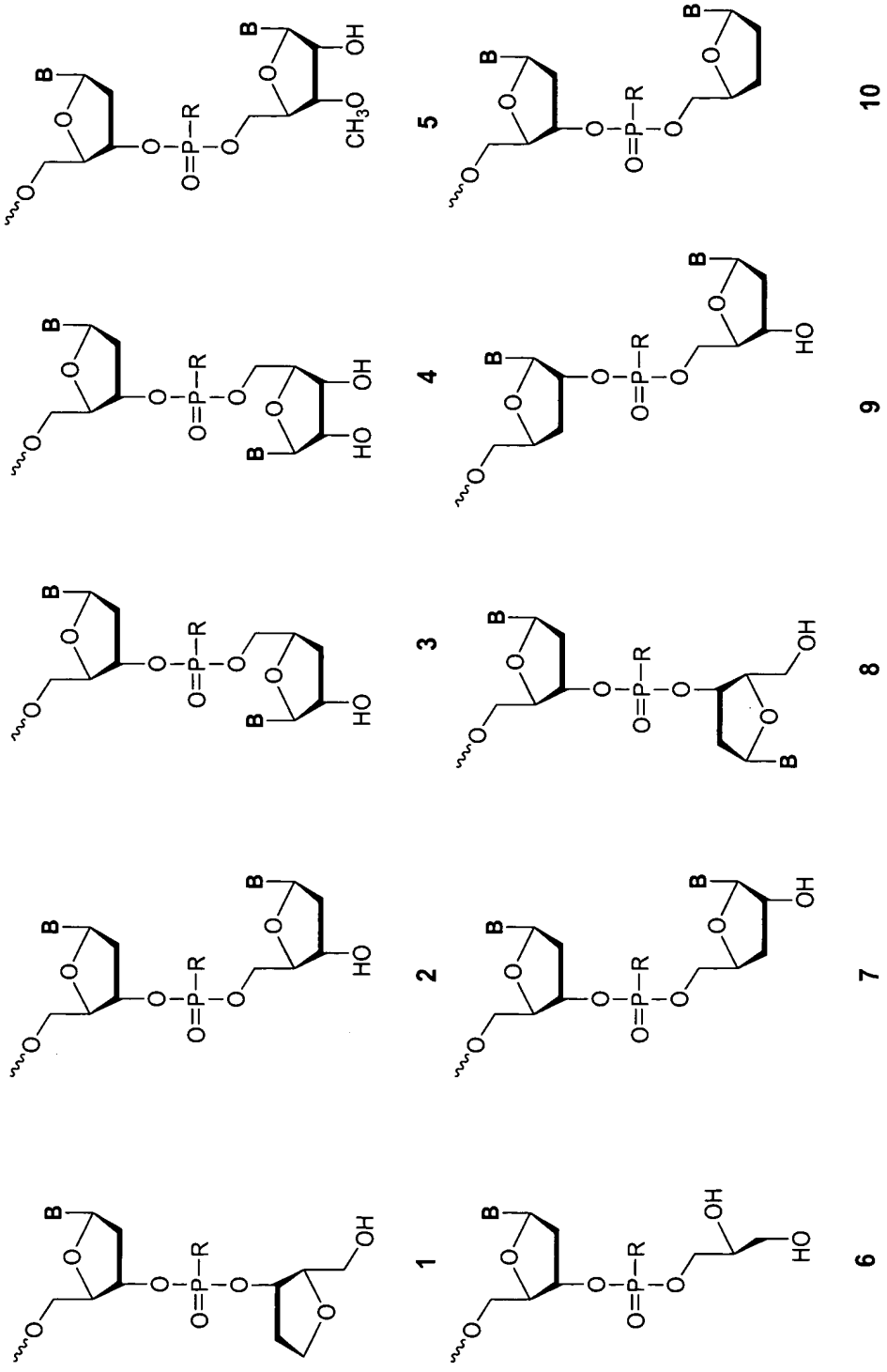


Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

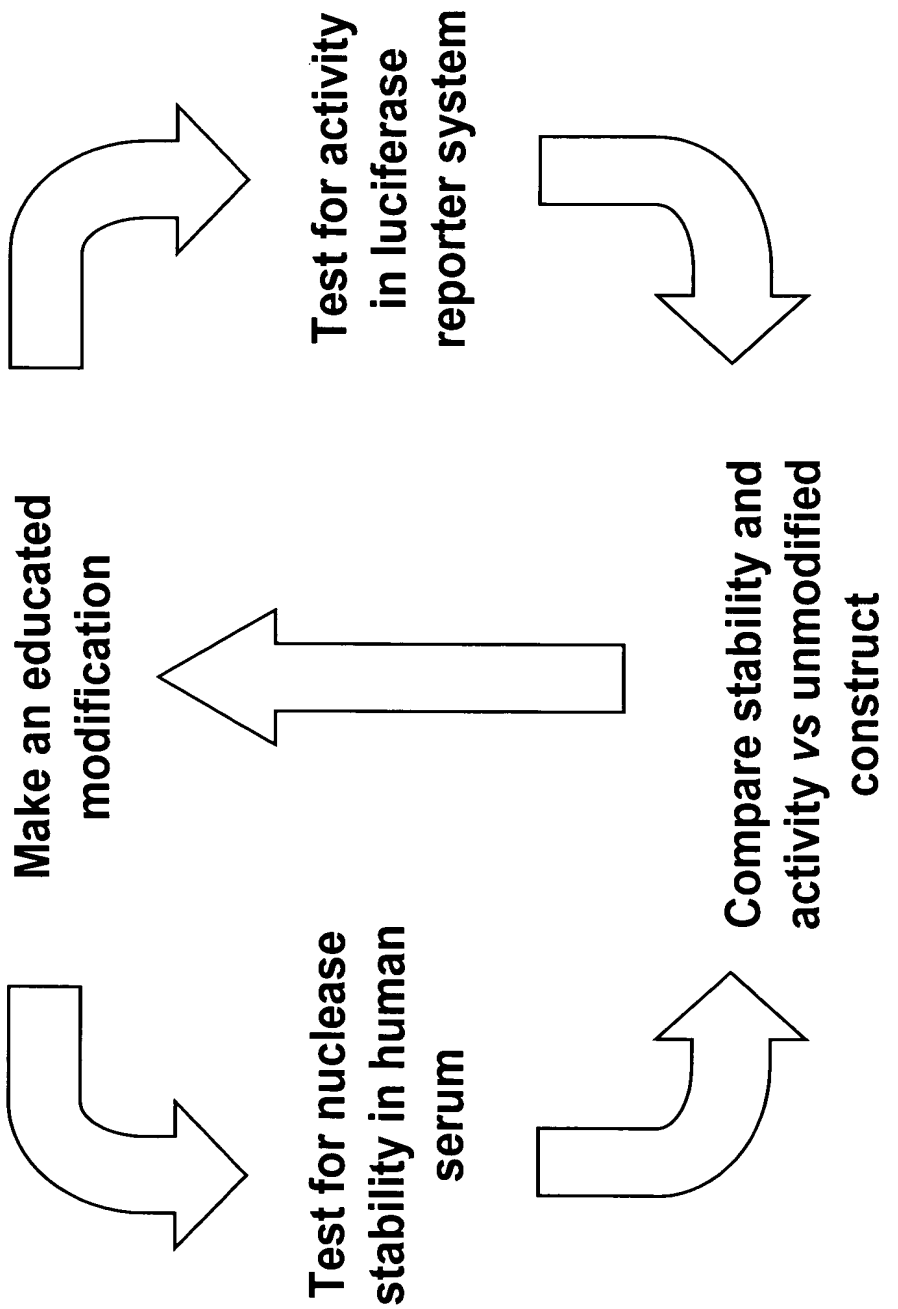
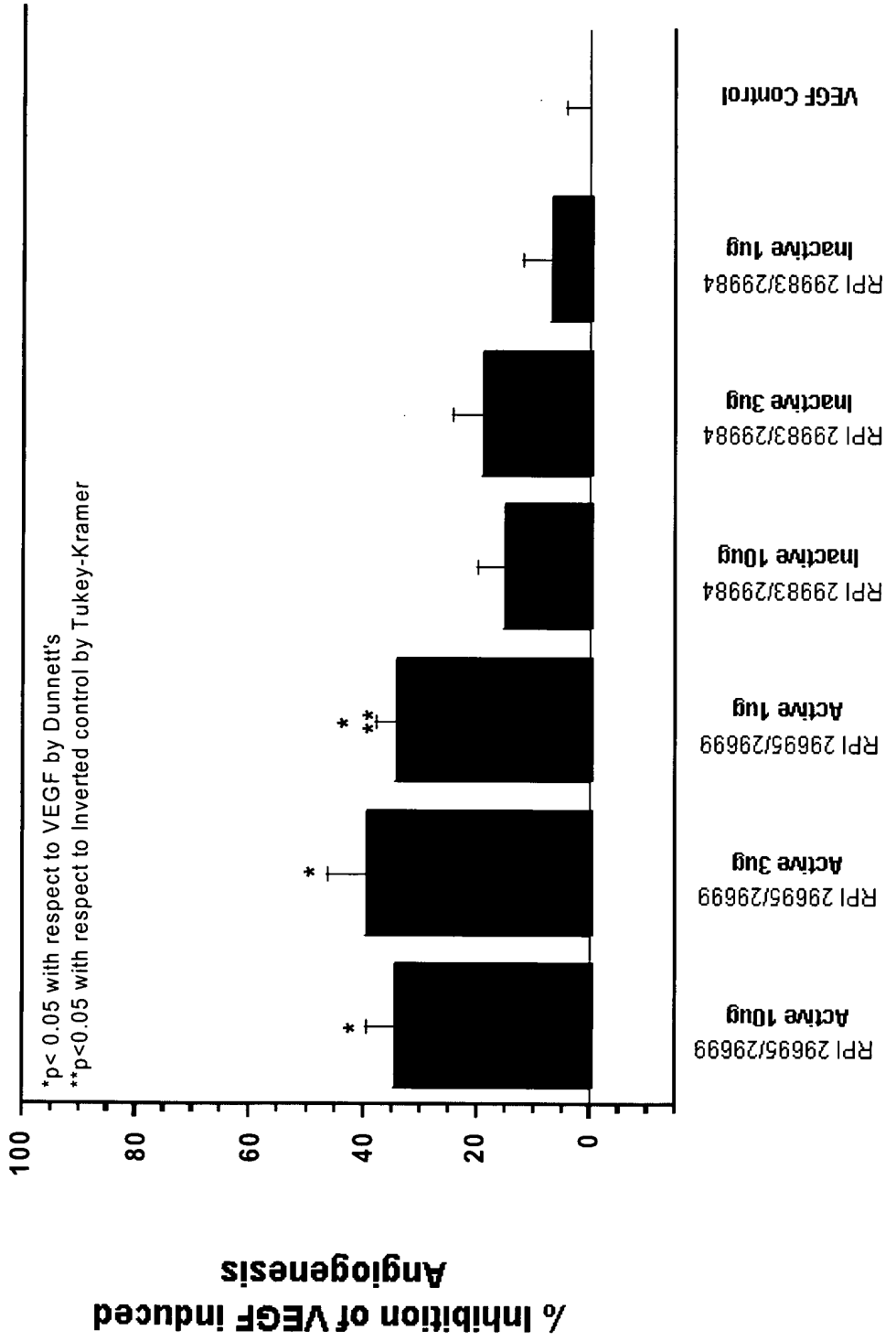


Figure 12: Inhibition of VEGF-Induced Angiogenesis by siRNAs



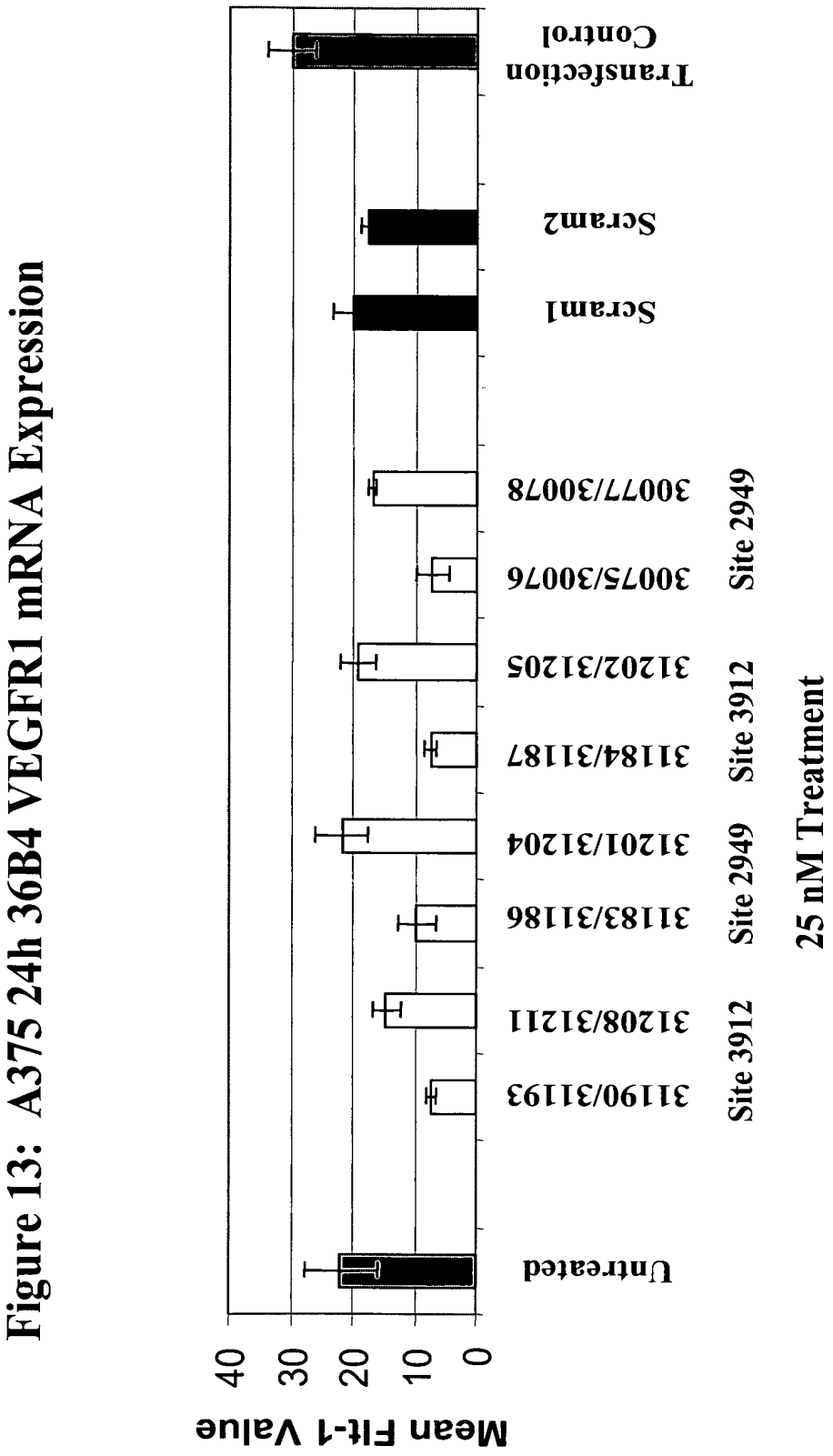
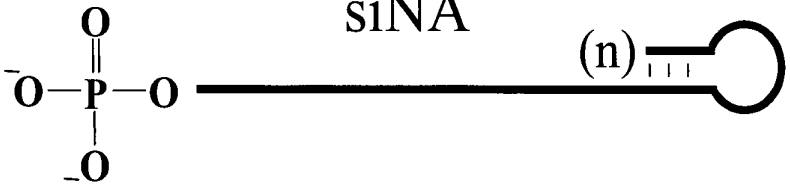


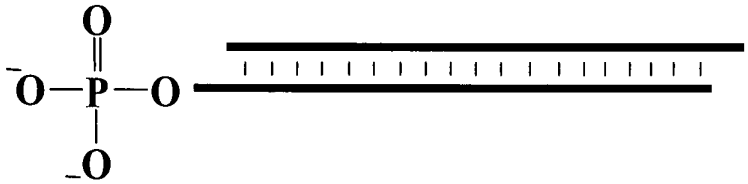
Figure 13: A375 24h 36B4 VEGFR1 mRNA Expression
Mean Flt-1 Value
40
30
20
10
0
Untreated
31190/31193
31208/31211
31183/31186
31201/31204
31184/31187
31202/31205
30075/30076
30077/30078
Scram1
Scram2
Transfection Control
Site 3912
Site 2949
Site 3912
Site 2949
25 nM Treatment

Figure 14: Phosphorylated siNA constructs

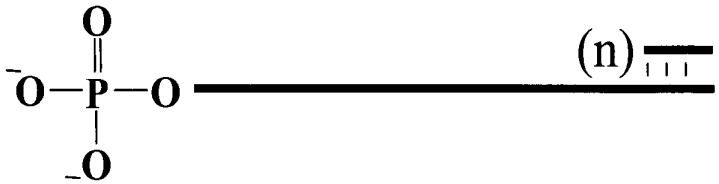
Phosphates can be modified as described herein

Asymmetric hairpin siNA





Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

Figure 15: 5'-phosphate modifications

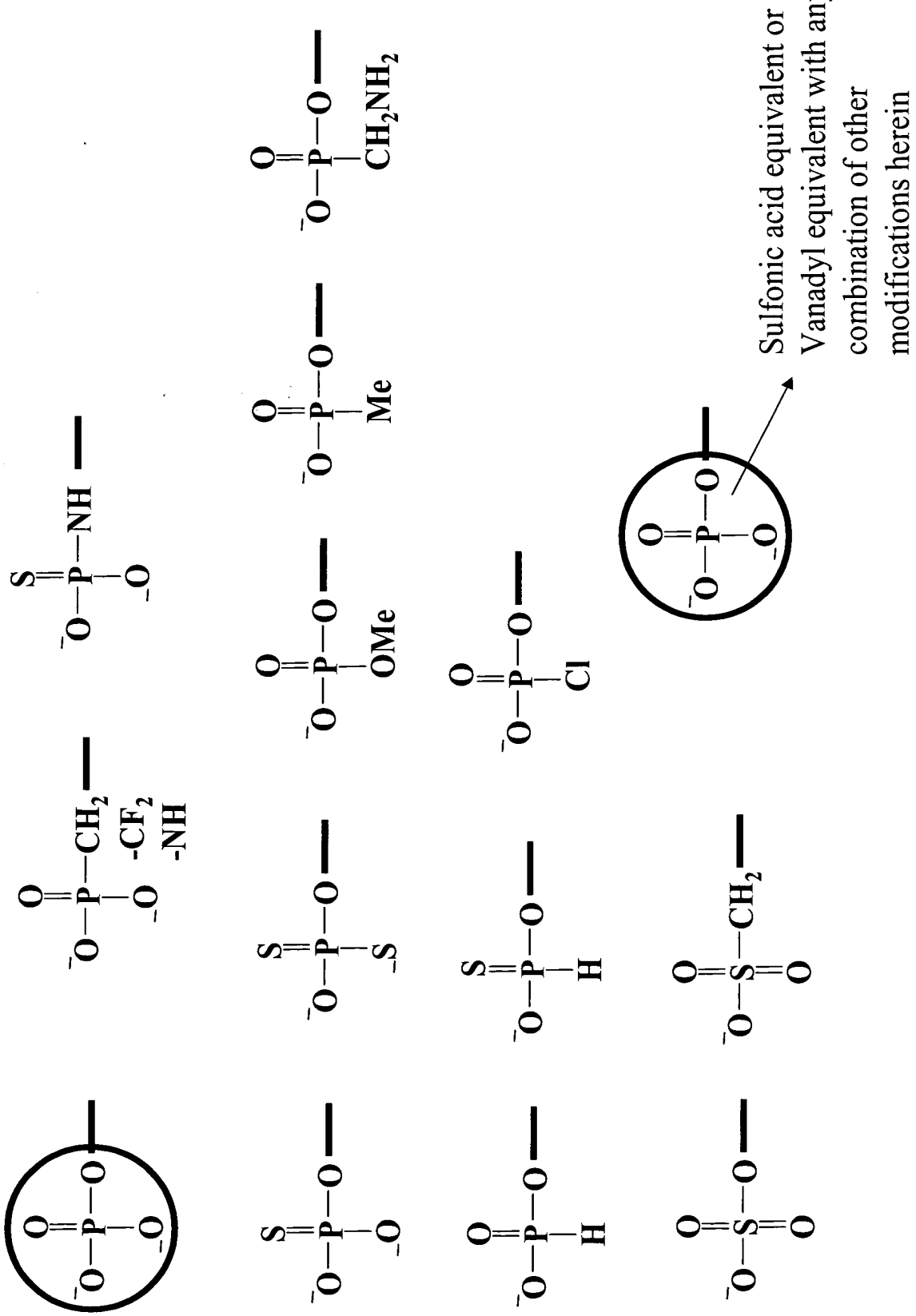
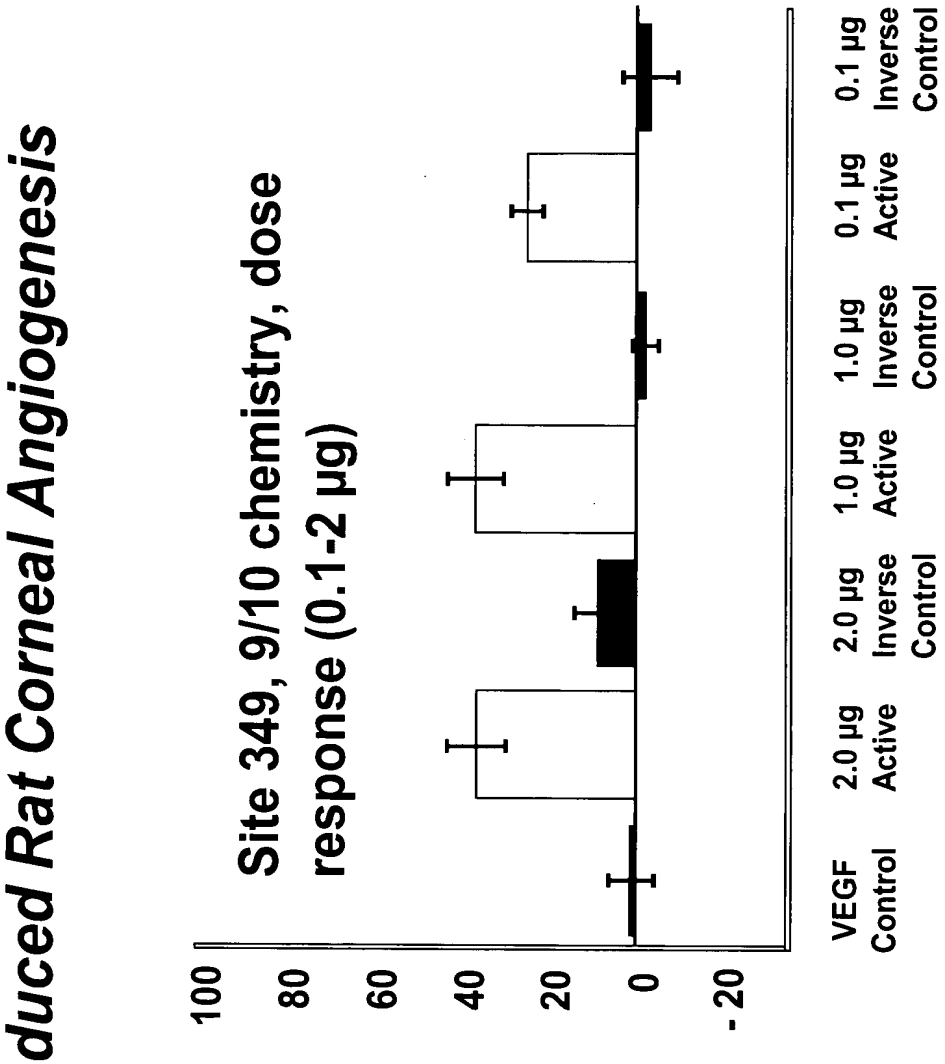


Figure 16: siNA Targeting VEGFR-1 Inhibits VEGF-Induced Rat Corneal Angiogenesis



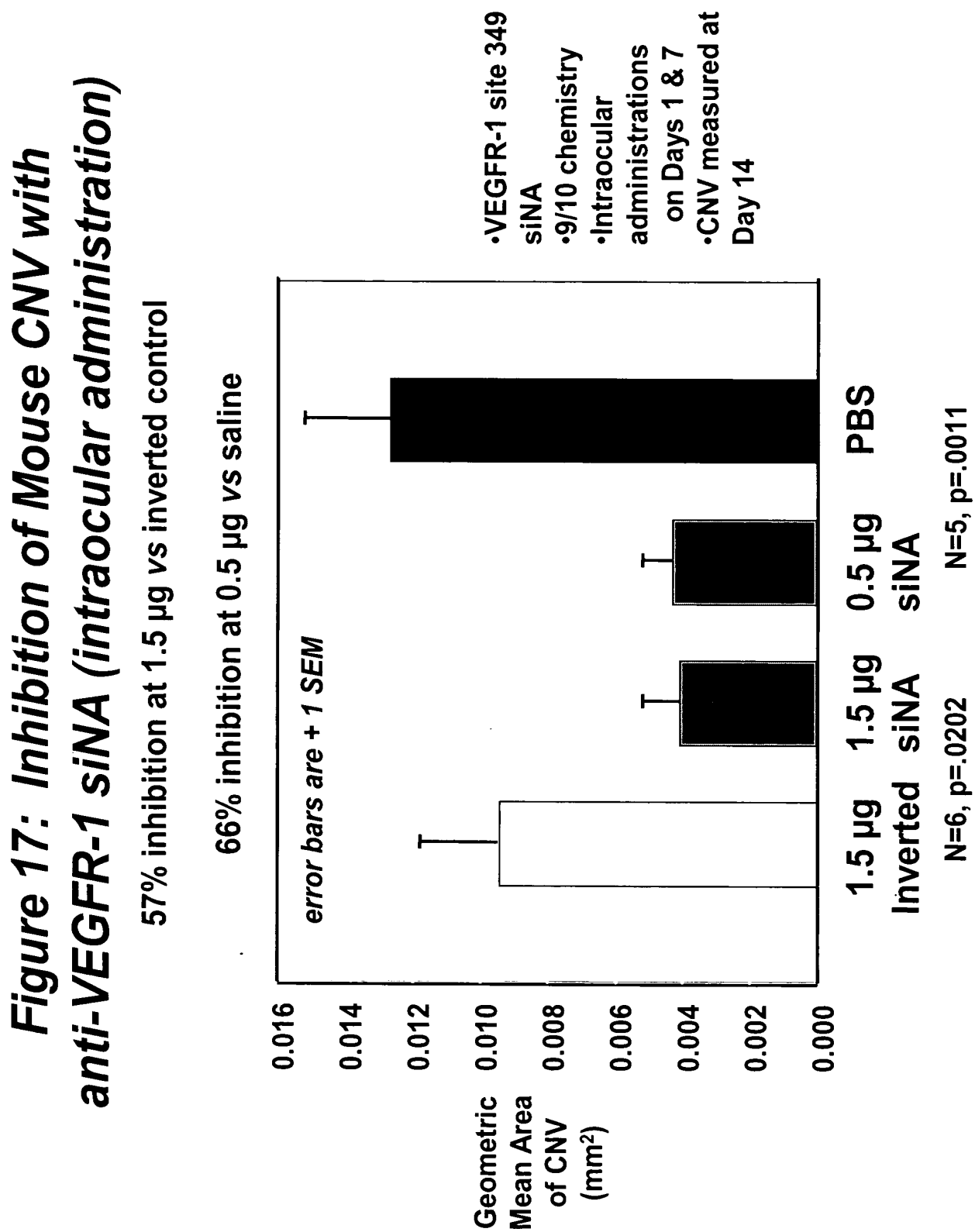


Figure 17: Inhibition of Mouse CNV with anti-VEGFR-1 siNA (intraocular administration)
57% inhibition at 1.5 µg vs inverted control
66% inhibition at 0.5 µg vs saline
error bars are + 1 SEM
•VEGFR-1 site 349 siNA
•9/10 chemistry
•Intraocular administrations on Days 1 & 7
•CNV measured at Day 14
Geometric Mean Area of CNV (mm²)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
1.5 µg Inverted siNA
1.5 µg siNA
0.5 µg siNA
PBS
N=6, p=.0202
N=5, p=.0011

Figure 18: Inhibition of Mouse CNV with anti-VEGFR-1 siNA (periocular administration)

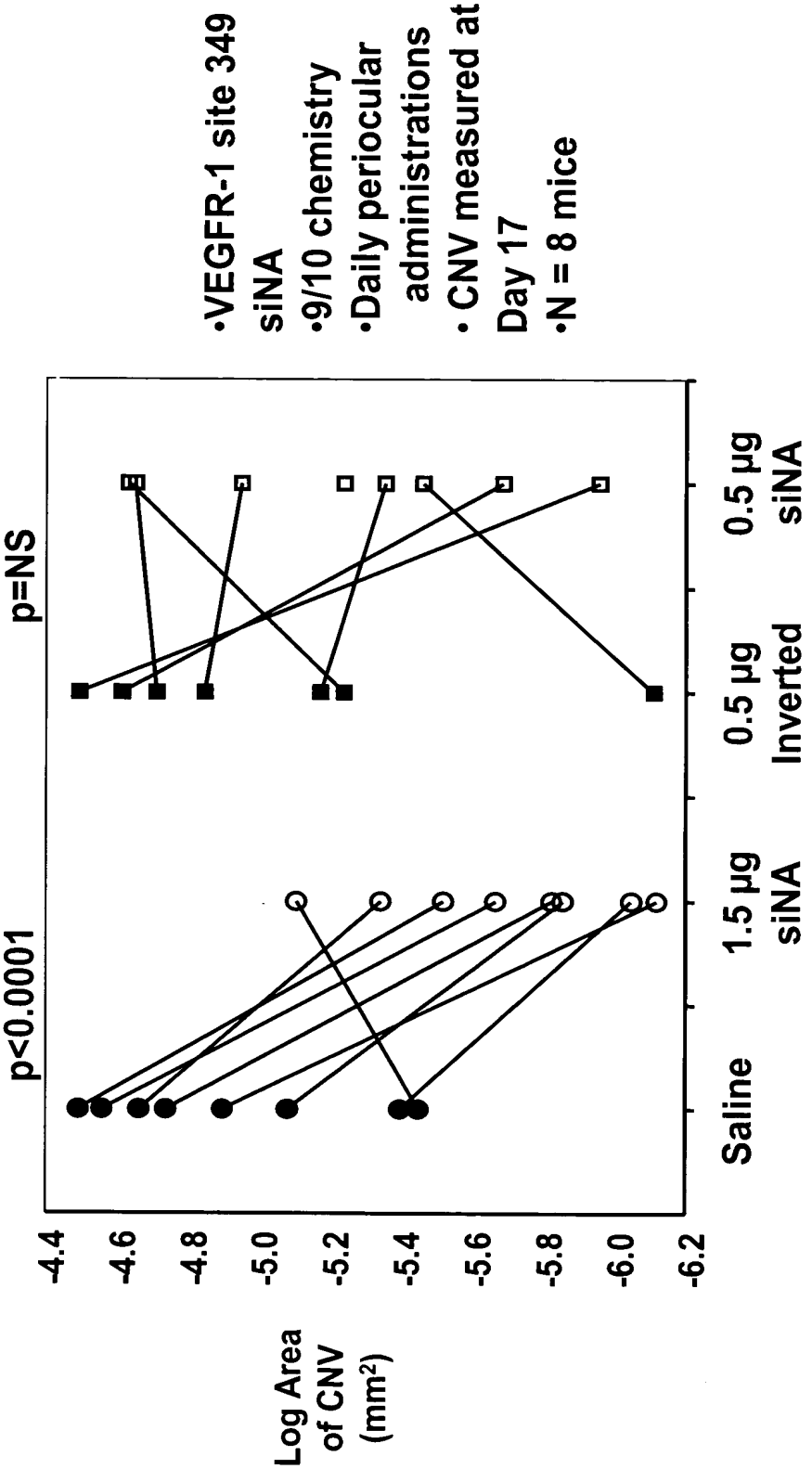
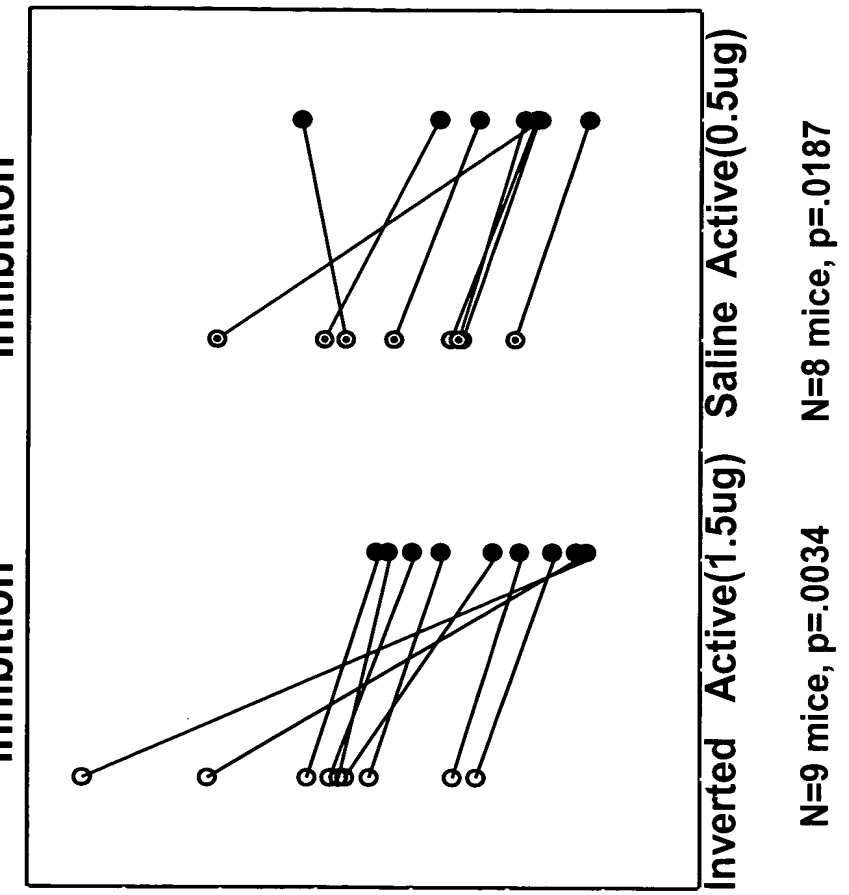


Figure 19: Inhibition of Mouse CNV with anti-VEGFR-1 siNA (periocular administration)



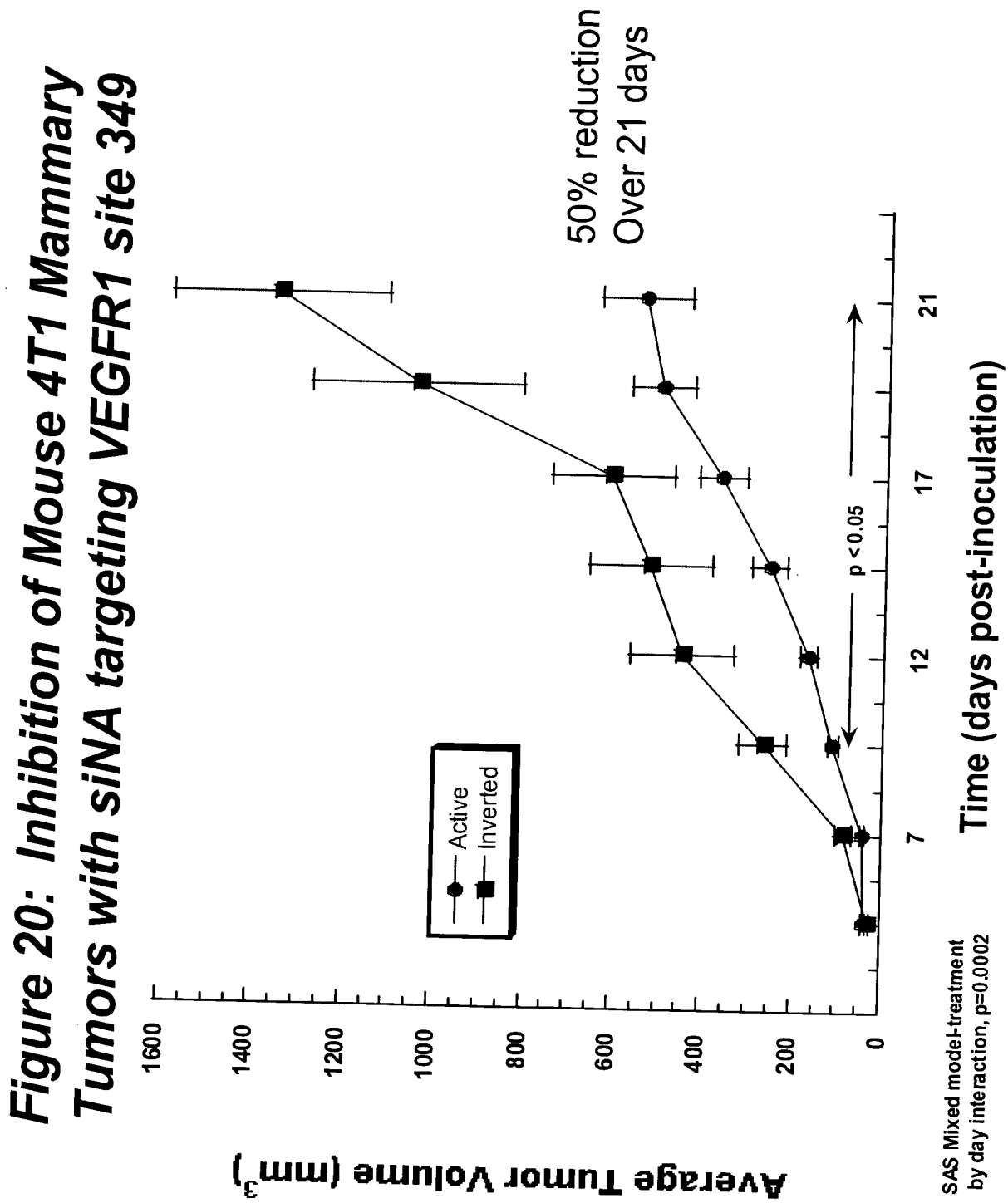


Figure 20: Inhibition of Mouse 4T1 Mammary Tumors with siNA targeting VEGFR1 site 349
50% reduction
Over 21 days
Active
Inverted
p < 0.05
Average Tumor Volume (mm3)
Time (days post-inoculation)
0
200
400
600
800
1000
1200
1400
1600
7
12
17
21
SAS Mixed model-treatment
by day interaction, p=0.0002

Figure 21: Inhibition of Mouse 4T1 Mammary Tumors with siNA targeting VEGFR1 site 349 Decreased level of Soluble VEGFr1

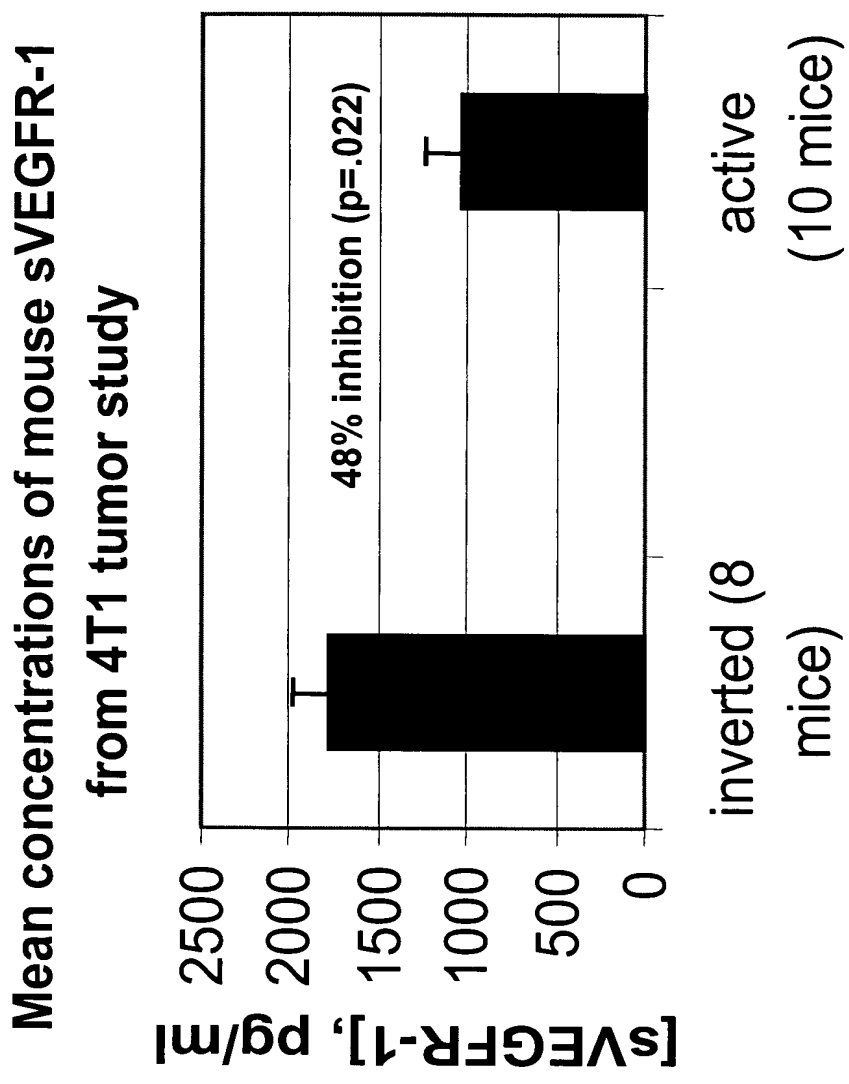
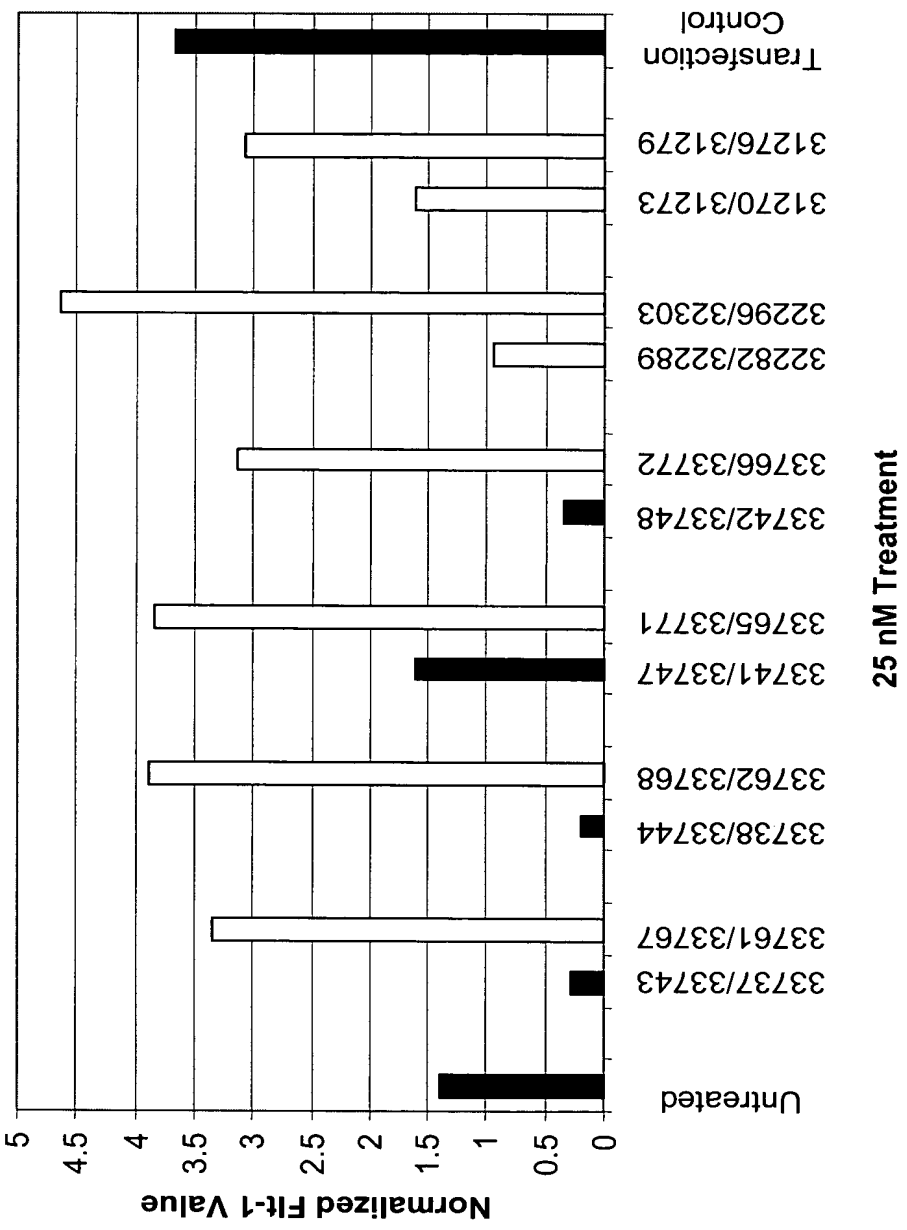


Figure 22A: Inhibition of VEGFR1 RNA expression with siNAs targeting VEGFR1 and VEGFR2 homologous sequences



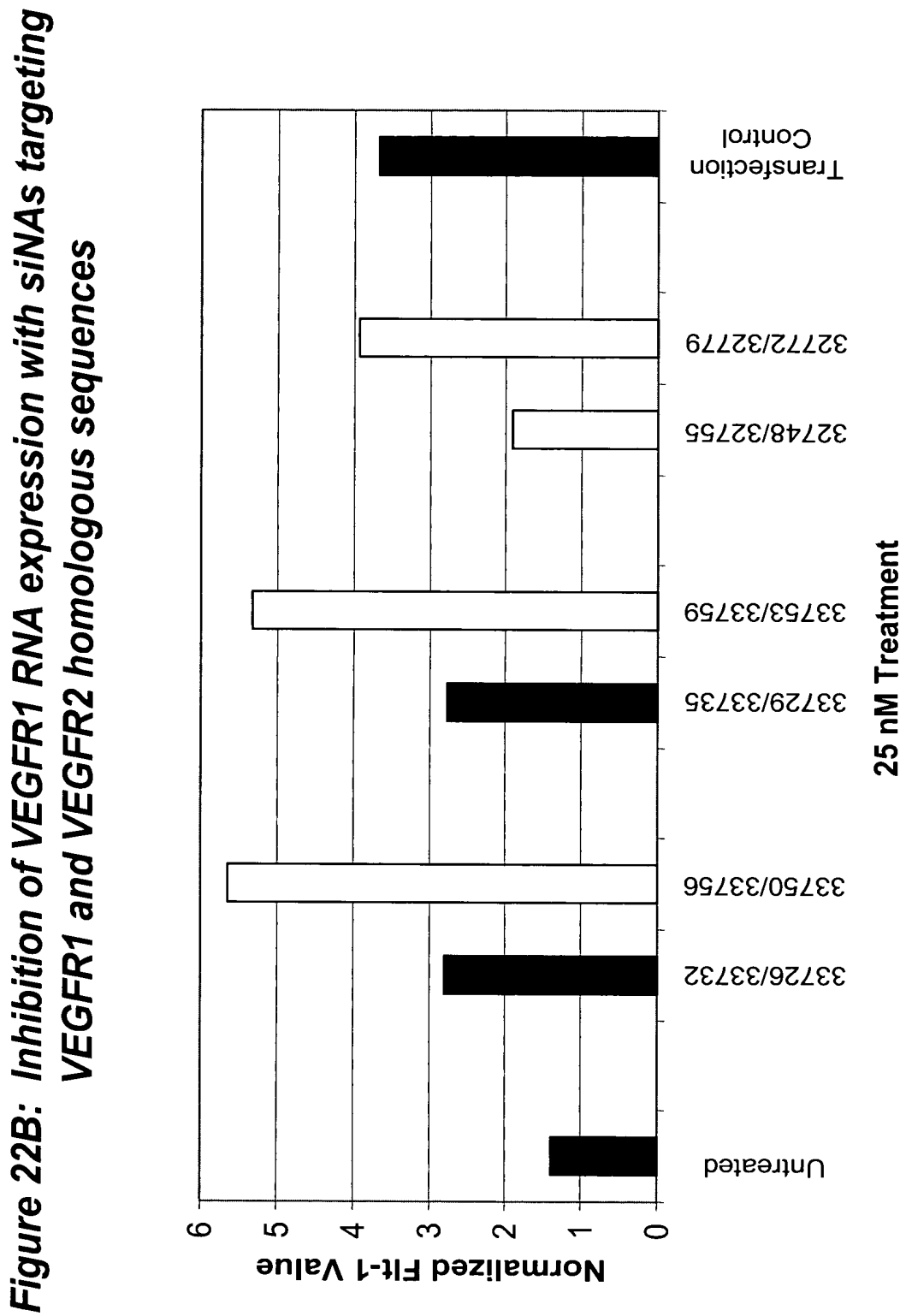


Figure 22B: Inhibition of VEGFR1 RNA expression with siNAs targeting VEGFR1 and VEGFR2 homologous sequences

Figure 23A: Inhibition of VEGFR2 RNA expression with siNAs targeting VEGFR1 and VEGFR2 homologous sequences

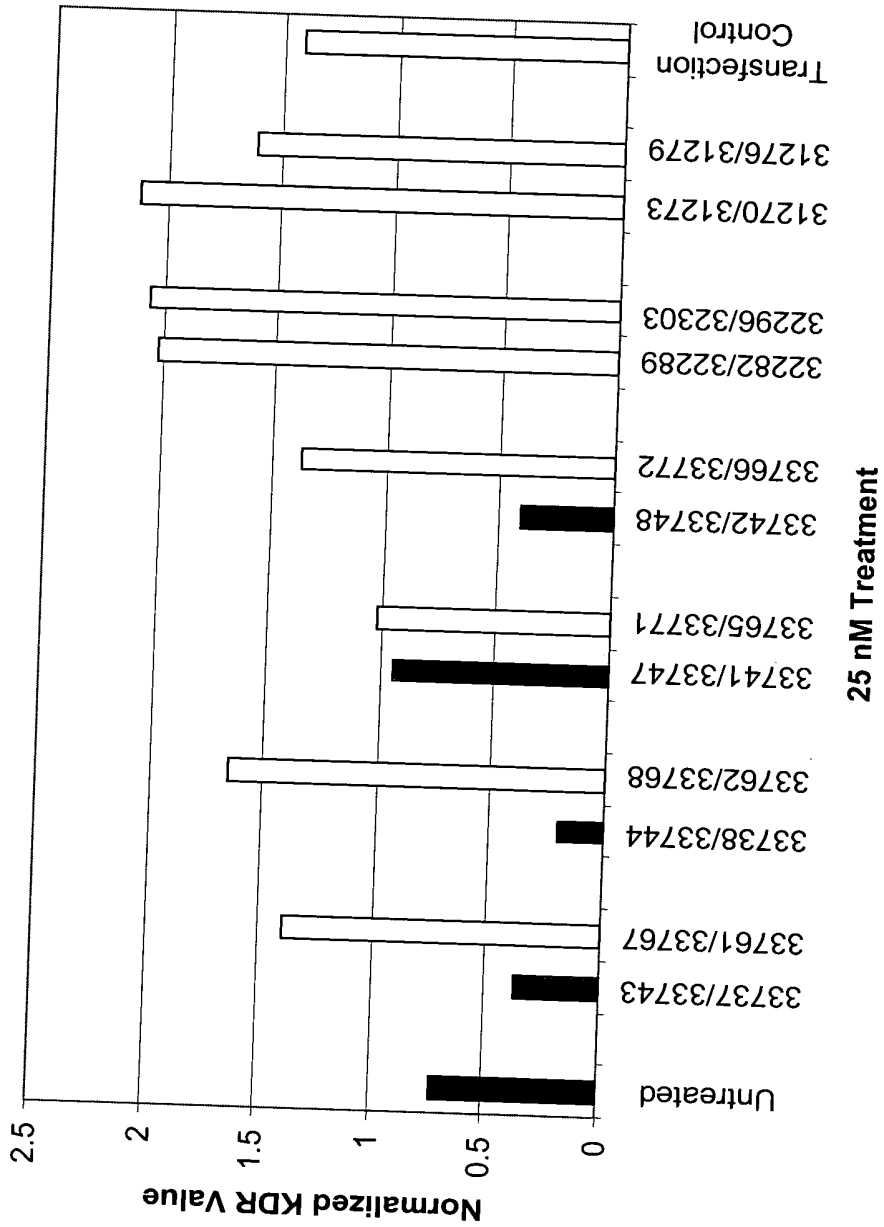
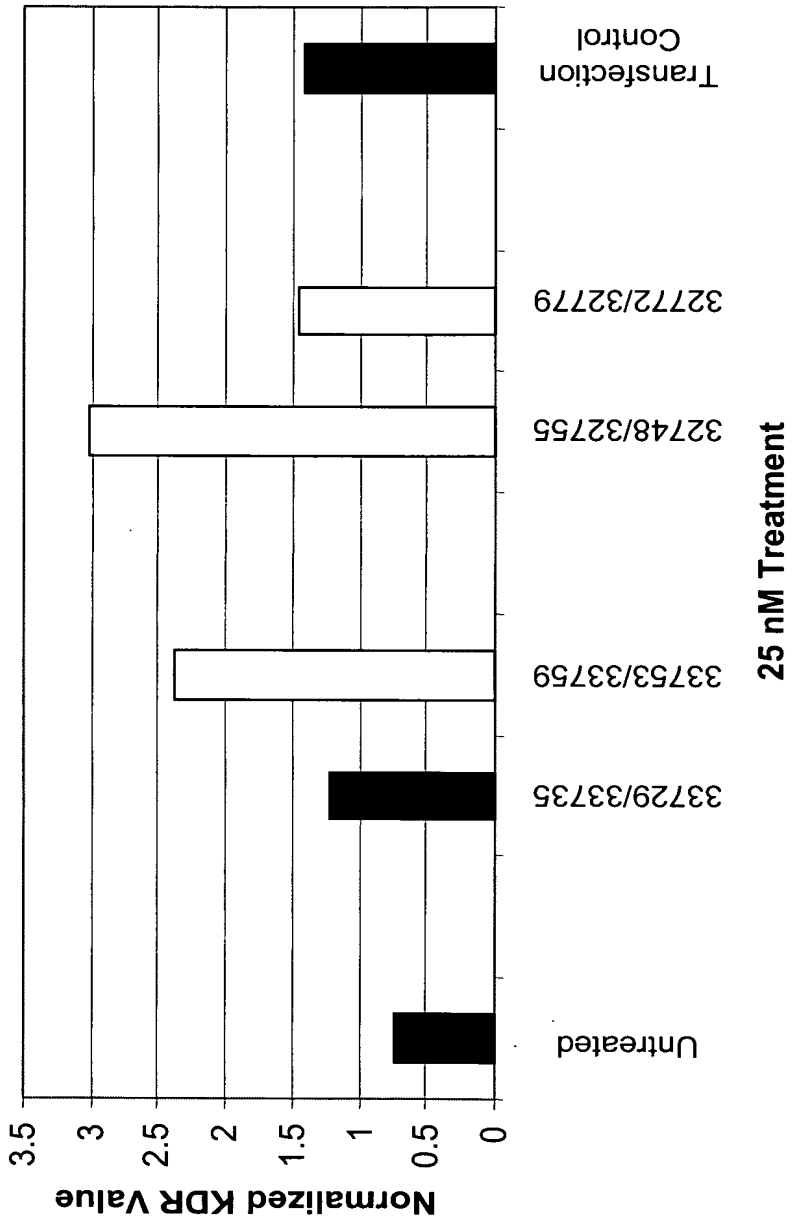


Figure 23B: Inhibition of VEGFR2 RNA expression with siNAs targeting VEGFR1 and VEGFR2 homologous sequences



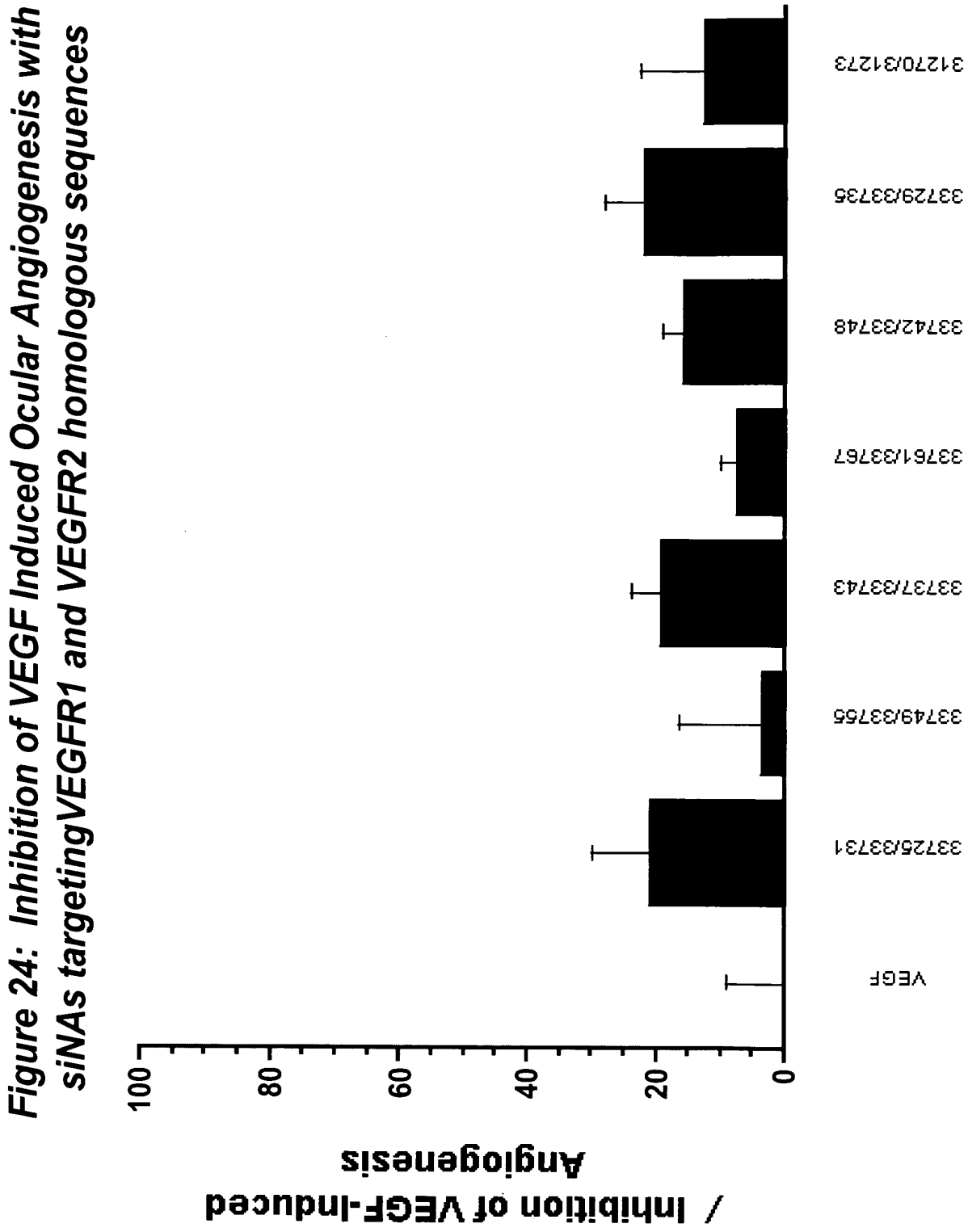


Figure 24: Inhibition of VEGF Induced Ocular Angiogenesis with siNAs targetingVEGFR1 and VEGFR2 homologous sequences